Supporting Information

TGN-020 Reference Synthesis

Reagents were purchased from Aldrich Chemical Company (St. Louis, MO, USA), Wako Pure Chemical Industries (Osaka, Japan) or Tokyo Chemical Industries (Tokyo, Japan) at the highest purity available and were used without further purification. Additional solvents were obtained from Nacalai Tesque (Tokyo, Japan) and were used as obtained. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury 300 instrument in DMSO-D_6, referenced to internal TMS (0 ppm), or D_2O, referenced to the HOD peak (4.8 ppm), as indicated. Routine HPLC chromatograms were recorded on a Waters Delta 600 instrument (Waters, Milford, MA, USA) fitted with a model 2998 detector using an Atlantis column (4.6x100 mm). Mass spectra were obtained on a Waters LCT Premier XE mass spectrometer (Waters, Milford MA, USA). Elemental analysis was determined from a solid sample using a Perkin-Elmer 2400 series II CHN analyzer.[1]

TGN-020·HCl: N-methylmorpholine (3.5 mL, 31.9 mL) was added to an ice-bath chilled solution of 2-amino-1,3,4-thiadiazole (1.00 g, 9.90 mmol) in dichloromethane (50 mL) under a nitrogen atmosphere. The resulting mixture was stirred for 10 minutes, following which, solid nicotinoyl chloride hydrochloride (1.98 g, 11.1 mmol) was added. The resulting solution was allowed to warm to room temperature and was stirred under N_2 for a further 12 h. Solvent was removed *in vacuo*, and the resulting solid was washed with ether (2 x 50 mL), followed by, water (3 x 50 mL). The resulting white solid was recrystallized from hot

[1] Professor Takeshi Takahashi (Tokyo Institute of Technology) is thanked for his assistance in obtaining elemental analysis data.

aqueous HCl (0.5 M, 100 mL). Upon cooling, the resultant white precipitate was filtered and dried *in vacuo* to give the desired product as its HCl salt (1.98 g, 8.20 mmol). ^{1}H-NMR (DMSO), HPLC (5-95% methanol gradient in water, 9.3 min), MS (Calculated: 207.0 m+H; Found: 207.2 m+H) and elemental analysis (Anal. Calculated for $C_8H_7N_4ClOS \bullet 0.25\ H_2O$: C, 38.87; H, 3.06; N, 22.67: Found: C, 38.95; H, 2.76; N, 22.88) were consistent with the assigned structure.

TGN-020-Na^{+}: TGN-020·HCl (1.34 g, 5.54 mmol) was neutralized by $NaHCO_3$ (0.595 g, 7.09 mmol) dissolved in 65 - 75 °C water (100 mL). The resulting water insoluble solid was washed with water (3 x 100 mL), then suspended in water (100 mL), to which 1 M NaOH (5.26 mL, 5.26 mmol) was slowly added portion wise until only faint turbidity remained. The resulting mixture was stirred for 30 min, and then clarified by filtration through a packed glass wool column. The clear filtrate was lyophilized to yield the desired compound as a white powder (0.999 g, 4.41 mmol), which was used as obtained. ^{1}H NMR (D_2O) and HPLC (isocratic, 3% aqueous MeOH) were consistent with the assigned structure.

PET Time Course Profiles

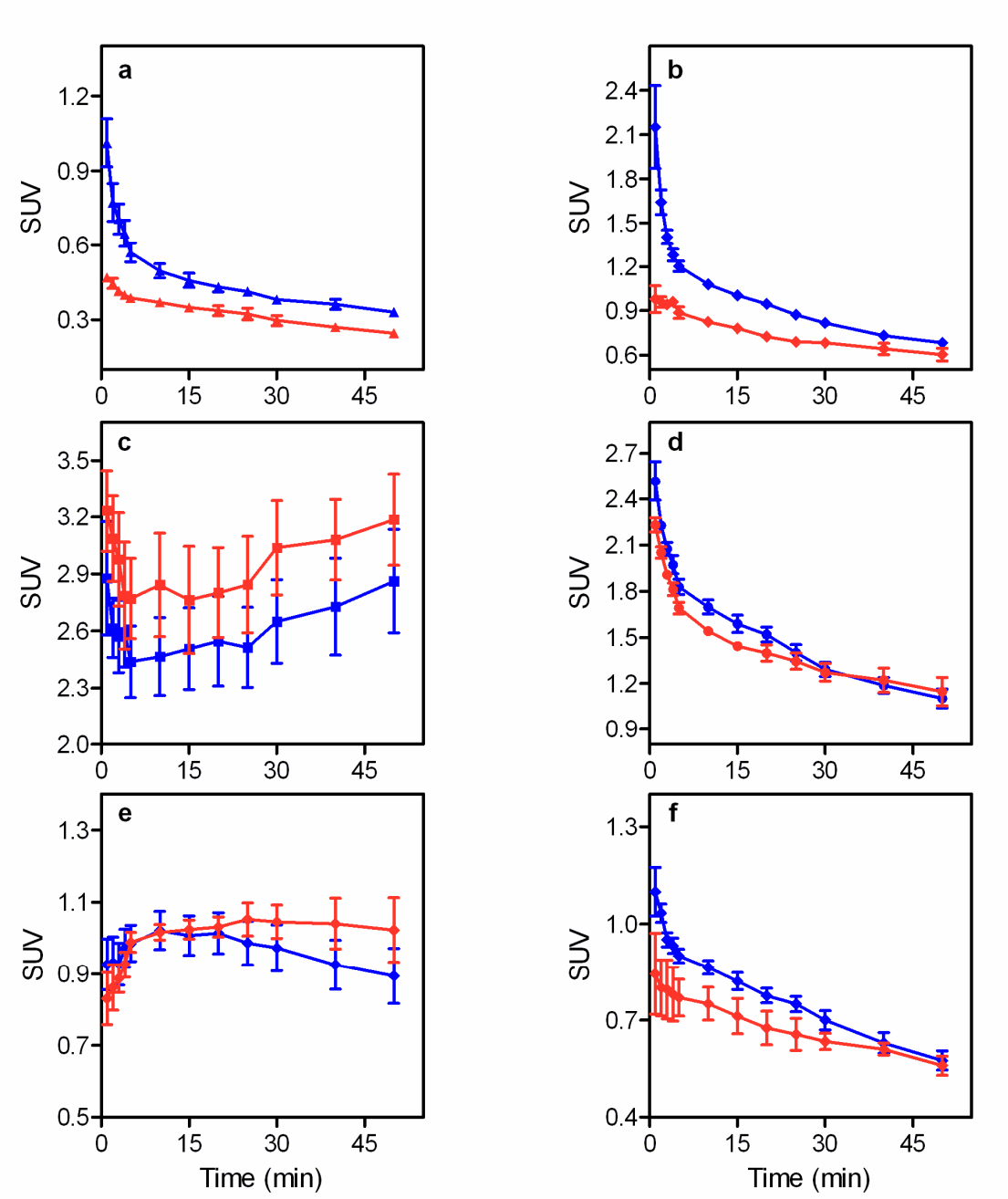

Figure S-1. [^{11}C]TGN-020 uptake time-course plots for all tissues sampled from PET imaging data. (a) brain, (b) skeletal muscle, (c) liver, (d) heart, (e) eye, (f) lungs. Data obtained from WT mice (n = 6, blue line) and KO mice (n = 4, red line) are shown. P values: (single tail T-test) 0.003, 0.007, <0.0001, 0.2, 0.3 and 0.02, respectively for a - f.

Table S-1. [^{11}C]TGN-020 SUV ± SEM at selected time points taken from WT and KO animal PET data.

	Brain		Muscle		Liver		Heart		Eye		Lungs	
Time (min)	WT[1]	KO[2]	WT	KO	WT	KO	WT	KO	WT	KO	WT	KO
1.0	1.01 ± 0.1	0.47 ± 0.02	2.15 ± 0.3	0.98 ± 0.09	2.88 ± 0.3	3.23 ± 0.2	2.52 ± 0.1	2.23 ± 0.05	0.93 ± 0.07	0.83 ± 0.07	1.10 ± 0.07	0.84 ± 0.1
2.0	0.77 ± 0.07	0.44 ± 0.02	1.64 ± 0.1	0.96 ± 0.03	2.62 ± 0.2	3.08 ± 0.2	2.23 ± 0.03	2.05 ± 0.04	0.93 ± 0.07	0.86 ± 0.06	1.03 ± 0.03	0.80 ± 0.09
3.0	0.70 ± 0.06	0.42 ± 0.02	1.40 ± 0.05	0.94 ± 0.01	2.57 ± 0.2	2.98 ± 0.2	2.07 ± 0.04	1.91 ± 0.01	0.93 ± 0.05	0.89 ± 0.04	0.95 ± 0.02	0.80 ± 0.09
4.0	0.65 ± 0.05	0.40 ± 0.01	1.28 ± 0.04	0.96 ± 0.01	2.60 ± 0.2	2.78 ± 0.3	1.97 ± 0.06	1.81 ± 0.04	0.97 ± 0.05	0.93 ± 0.03	0.93 ± 0.02	0.78 ± 0.08
5.0	0.57 ± 0.04	0.39 ± 0.01	1.20 ± 0.03	0.89 ± 0.04	2.44 ± 0.2	2.77 ± 0.2	1.83 ± 0.05	1.69 ± 0.04	0.98 ± 0.05	0.99 ± 0.03	0.90 ± 0.02	0.77 ± 0.06
10.0	0.50 ± 0.03	0.37 ± 0.01	1.08 ± 0.01	0.83 ± 0.02	2.46 ± 0.2	2.84 ± 0.3	1.70 ± 0.04	1.54 ± 0.02	1.02 ± 0.05	1.02 ± 0.02	0.86 ± 0.02	0.75 ± 0.05
15.0	0.46 ± 0.03	0.35 ± 0.01	1.01 ± 0.01	0.78 ± 0.02	2.51 ± 0.2	2.76 ± 0.3	1.59 ± 0.06	1.44 ± 0.02	1.01 ± 0.05	1.02 ± 0.03	0.82 ± 0.03	0.71 ± 0.05
20.0	0.43 ± 0.02	0.34 ± 0.02	0.95 ± 0.01	0.72 ± 0.01	2.55 ± 0.2	2.80 ± 0.2	1.52 ± 0.05	1.40 ± 0.05	1.01 ± 0.06	1.03 ± 0.03	0.78 ± 0.02	0.68 ± 0.05
25.0	0.41 ± 0.02	0.32 ± 0.02	0.87 ± 0.02	0.69 ± 0.02	2.51 ± 0.2	2.84 ± 0.2	1.40 ± 0.05	1.34 ± 0.05	0.99 ± 0.06	1.05 ± 0.04	0.75 ± 0.02	0.66 ± 0.05
30.0	0.38 ± 0.01	0.30 ± 0.02	0.82 ± 0.02	0.68 ± 0.02	2.65 ± 0.2	3.04 ± 0.2	1.29 ± 0.04	1.27 ± 0.06	0.97 ± 0.06	1.04 ± 0.04	0.70 ± 0.03	0.63 ± 0.02
40.0	0.36 ± 0.02	0.27 ± 0.02	0.73 ± 0.03	0.64 ± 0.04	2.73 ± 0.2	3.08 ± 0.2	1.19 ± 0.05	1.22 ± 0.08	0.92 ± 0.07	1.04 ± 0.07	0.63 ± 0.03	0.61 ± 0.02
50.0	0.33 ± 0.01	0.25 ± 0.01	0.68 ± 0.02	0.60 ± 0.04	2.86 ± 0.3	3.19 ± 0.2	1.10 ± 0.06	1.15 ± 0.09	0.89 ± 0.08	1.02 ± 0.09	0.58 ± 0.03	0.56 ± 0.03

1. SUV for wild type (WT) models averaged from $n = 6$ mice.
2. SUV for AQP4 null (KO) models averaged from $n = 4$ mice.